

FILE 'REGISTRY' ENTERED AT 11:21:09 ON 28 OCT 2005

=> S RESTRICTION ENZYME/CN

L1 1 RESTRICTION ENZYME/CN

FILE 'CAPLUS' ENTERED AT 11:21:25 ON 28 OCT 2005

=> S RESTRICTION(W) (ENDONUCLEASE OR ENZYME); S L1; S L2, L3

98771 RESTRICTION
12971 RESTRICTIONS
110924 RESTRICTION
(RESTRICTION OR RESTRICTIONS)
27082 ENDONUCLEASE
8111 ENDONUCLEASES
31434 ENDONUCLEASE
(ENDONUCLEASE OR ENDONUCLEASES)
753212 ENZYME
435596 ENZYMES
951933 ENZYME
(ENZYME OR ENZYMES)

L2 32185 RESTRICTION(W) (ENDONUCLEASE OR ENZYME)

L3 3936 L1

L4 32499 (L2 OR L3)

=> S MMEI OR MME1 OR (MME(W) (I OR 1))

46 MMEI
1 MME1
700 MME
24 MMES
717 MME
(MME OR MMES)

4127935 I

8443290 1

32 MME(W) (I OR 1)

L5 78 MMEI OR MME1 OR (MME(W) (I OR 1))

=> S L5 AND L5

L6 78 L5 AND L5

=> D 1-78 TI

=> D 22,46,49,59 CBIB ABS

L6 ANSWER 22 OF 78 CAPLUS COPYRIGHT 2005 ACS on STN

2004:60631 Document No. 140:124553 Method for producing recombinant
Methylophilus methylotrophus type II restriction endonucleases,
MmeI with methyltransferase activity. Morgan, Richard D.; Bhatia,
Tanya; Davis, Theodore; Lovasco, Lindsay (New England Biolabs, Inc., USA).

PCT Int. Appl. WO 2004007670 A2 20040122, 61 pp. DESIGNATED STATES: W:

AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR,
CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID,
IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD,
SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA,
ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, RW: AT, BE, BF, BJ, CF, CG, CH,
CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE,
NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO
2003-US21570 20030710. PRIORITY: US 2002-2002/PV395431 20020712.

AB The present invention provides a DNA (DNA) fragment which encodes the MmeI type
II restriction endonuclease enzyme. This one polypeptide possesses two related

enzymic functions; namely an endonuclease activity which recognizes the DNA sequence 5'-TCC(Pu)AC-3' and cleaves as indicated by the arrows: 5' -TCCRAC (N20) ↓-3' 3'-AGGYTG(N18)↑-5' and a second enzymic activity that recognizes the same DNA sequence, 5'-TCC(Pu)AC-3', but modifies this sequence by the addition of a Me group to prevent cleavage by the *MmeI* endonuclease activity.

L6 ANSWER 46 OF 78 CAPLUS COPYRIGHT 2005 ACS on STN

1999:28358 Document No. 130:233897 Two intertwined methylation activities of the *MmeI* restriction-modification class-IIS system from *Methylophilus methylotrophus*. Tucholski, Janusz; Zmijewski, Jaroslaw W.; Podhajska, Anna J. (Faculty of Biotechnology, Department of Biotechnology, University of Gdansk and Medical University of Gdansk, Gdansk, 80-822, Pol.). *Gene*, 223(1-2), 293-302 (English) 1998. CODEN: GENED6. ISSN: 0378-1119. Publisher: Elsevier Science B.V..

AB The class-IIS restriction endonuclease, *R.MmeI*, was isolated from *Methylophilus methylotrophus*. It was originally described as a monomeric enzyme, with the native Mr 105000±7000, which did not cleave DNA efficiently [Boyd et al. (1986) *Nucleic Acids Res.* 14, 5255-5274; Tucholski et al. (1995) *Gene* 157, 87-92]. However, it was discovered that *R.MmeI* endonucleolytic activity is enhanced by S-adenosyl-L-methionine (AdoMet) and sinefungin, an analog of AdoMet. Surprisingly, the purified *R.MmeI* endonuclease was found to have a second enzymic activity, namely methylation of the adenine residue to N6-methyladenine in the top strand of the *MmeI*-recognition sequence, 5'-TCCR*AC-3' (*A=meA). The *R.MmeI* methylating activity requires AdoMet and is increased in the presence of several divalent cations, 20-fold by Mg²⁺ or Ca²⁺, and less by Mn²⁺, Zn²⁺ and Co²⁺; however, methylation is inhibited entirely by sinefungin, at concns. above 9 µM. The latter observation shows that the enhancing effect of AdoMet or sinefungin on the DNA cleavage was not related to the process of DNA methylation. Furthermore, a second component of the *MmeI* restriction-modification system, a *M.MmeI* methyltransferase, was isolated and purified. The *M.MmeI* protein was found to have an Mr of 48000±2000 (under denaturing conditions) and to methylate both adenine residues (*A) in the *MmeI*-recognition sequence 5'-TCCR*AC-3'/3'-*AGGYTG-5'. Methylation of the top strand does not inhibit the DNA cleavage by *R.MmeI*, whereas methylation of both DNA strands blocks the cleavage process.

L6 ANSWER 49 OF 78 CAPLUS COPYRIGHT 2005 ACS on STN

1995:680004 Document No. 123:136742 *MmeI*, a class-IIS restriction endonuclease: purification and characterization. Tucholski, Janusz; Skowron, Piotr M.; Podhajska, Anna J. (Department of Microbiology, University of Gdansk, Gdansk, 80-822, Pol.). *Gene*, 157(1/2), 87-92 (English) 1995. CODEN: GENED6. ISSN: 0378-1119. Publisher: Elsevier.

AB Two restriction endonucleases, *MmeI* and *MmeII*, from *Methylophilus methylotrophus* were purified to homogeneity. Both enzymes belong to the class-II restriction endonucleases (ENases) but exhibit very different enzymic and phys. properties. *MmeII* is a typical member of class-II ENases. It is a polymeric protein composed of 50-kDa subunits. In contrast to *MmeII*, *MmeI* is a monomeric protein of 101 kDa, cleaving a DNA mol. 20/18 nucleotides away from the asym. recognition sequence (5'-TCCRAC-3'); therefore, it is classified as a member of subclass-IIS. *MmeI* has an pI of 7.85 and is active in the pH range 6.5 to 10 with the optimum at 7 to 8. Increasing salt concentration creates an inhibitory effect on *MmeI*: 40 mM KCl decreases activity by 50%, 100 mM completely inhibits DNA cleavage. Tris·HCl (pH 7.5) at a concentration exceeding 20 mM inhibits *MmeI* activity. Mg²⁺ stimulates *MmeI* in the range of 0.2 to 35 mM, with the optimum between 0.5 and 10 mM.

L6 ANSWER 59 OF 78 CAPLUS COPYRIGHT 2005 ACS on STN

1986:567577 Document No. 105:167577 Isolation and computer-aided characterization of *MmeI*, a Type II restriction endonuclease from *Methylophilus methylotrophus*. Boyd, A. C.; Charles, I. G.; Keyte, J. W.; Brammar, W. J. (Dep. Biochem., Univ. Leicester, Leicester, LE1 7RH,

UK). Nucleic Acids Research, 14(13), 5255-74 (English) 1986. CODEN:
NARHAD. ISSN: 0305-1048.

AB A type II restriction endonuclease, MmeI, was purified from the obligate
methylophage, M. methylotrophus. The enzyme was shown to have a nonpalindromic
recognition sequence, TCCPuAC(N)20 (Pu = purine; N = nucleotide) and to cleave
the DNA 20 nucleotides 3' to the end of the recognition sequence-side. The
determination of the recognition sequence was achieved using 2 new computer
programs: RECOG, which predicts recognition sequences from the pattern of
restriction fragments obtained from DNAs of known sequence, and GELSIM, which
generates graphical simulations of DNA band patterns obtained by gel
electrophoresis of restriction digests of sequenced DNA mols.

=> E MORGAN R/AU

=> S E3,E75

77 "MORGAN R"/AU

45 "MORGAN RICHARD"/AU

L7 122 ("MORGAN R"/AU OR "MORGAN RICHARD"/AU)

=> E BHATIA T/AU

=> S E3-E9

7 "BHATIA T"/AU

2 "BHATIA T B"/AU

2 "BHATIA T K"/AU

1 "BHATIA T R"/AU

42 "BHATIA T S"/AU

9 "BHATIA TANIA"/AU

2 "BHATIA TANYA"/AU

L8 65 ("BHATIA T"/AU OR "BHATIA T B"/AU OR "BHATIA T K"/AU OR "BHATIA
T R"/AU OR "BHATIA T S"/AU OR "BHATIA TANIA"/AU OR "BHATIA TANYA
"/AU)

=> E DAVIS T/AU

=> S E3,E7,E11,E27-E29,E82-E85

49 "DAVIS T"/AU

1 "DAVIS T B"/AU

1 "DAVIS T F"/AU

8 "DAVIS T R"/AU

3 "DAVIS T R A"/AU

1 "DAVIS T R C"/AU

6 "DAVIS THEODORE"/AU

4 "DAVIS THEODORE B"/AU

8 "DAVIS THEODORE F"/AU

3 "DAVIS THEODORE R"/AU

L9 84 ("DAVIS T"/AU OR "DAVIS T B"/AU OR "DAVIS T F"/AU OR "DAVIS T
R"/AU OR "DAVIS T R A"/AU OR "DAVIS T R C"/AU OR "DAVIS THEODORE
"/AU OR "DAVIS THEODORE B"/AU OR "DAVIS THEODORE F"/AU OR "DAVIS
THEODORE R"/AU)

=> E LOVASCO/AU

=> S E5

L10 1 "LOVASCO LINDSAY"/AU

=> S L7,L8,L9,L10

L11 270 (L7 OR L8 OR L9 OR L10)

=> S L11 AND L4

L12 13 L11 AND L4

=> S L12 NOT L6

L13 12 L12 NOT L6

=> D 1-12 TI
=> D L13 5 CBIB ABS

L13 ANSWER 5 OF 12 CAPLUS COPYRIGHT 2005 ACS on STN

2005:36466 Document No. 142:109453 Novel type II restriction endonuclease, CstMI, obtainable from *Corynebacterium striatum* M82b. Morgan, Richard D.; Bhatia, Tanya (USA). U.S. Pat. Appl. Publ. US 2005009034 A1 20050113, 28 pp. (English). CODEN: USXXCO. APPLICATION: US 2003-616689 20030710.

AB In accordance with the present invention, there is provided a novel type II restriction endonuclease, obtainable from *Corynebacterium striatum* M82B, hereinafter referred to as "CstMI", which endonuclease recognizes the nucleotide sequence 5'-AAGGAG-3' in a double-stranded DNA mol. as shown below, 5'-AAGGAGN20↓-3' and 3'-TTCCTCN18↑-5', (wherein G represents guanine, C represents cytosine, A represents adenine, T represents thymine and N represents either G, C, A, or T). The new CstMI cleaves sequence at the phosphodiester bonds between the 20th and the 21th nucleotides 3' to the recognition sequence in the 5'-AAGGAG-3 strand of the DNA, and between the 18th and 19th nucleotides 5' to the recognition sequence in the complement stand, 5'-CTCCTT-3', to produce a 2 base 3' extension. The new CstMI also possesses a second enzymic activity that recognizes the same DNA sequence, 5'-AAGGAG-3', but modifies this sequence by the addition of a Me group to prevent cleavage by the CstMI endonuclease activity.

=> S MJAI
L14 4 MJAI

=> S L14 AND L4
L15 2 L14 AND L4

=> D 1-2 CBIB AB

L15 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2005 ACS on STN

2004:113494 Document No. 140:159654 Method for screening restriction endonucleases based on database homology searching of cognate DNA methylase sequences. Roberts, Richard J.; Byrd, Devon R.; Morgan, Richard D.; Patti, Jay; Noren, Christopher J. (New England Biolabs, Inc., USA). U.S. US 6689573 B1 20040210, 22 pp., Cont.-in-part of U.S. Ser. No. 486,356. (English). CODEN: USXXAM. APPLICATION: US 2000-577528 20000524. PRIORITY: US 1999-PV135541 19990524; US 2000-2000/486356 20000225.

AB The present invention relates to a novel method for screening and identifying restriction endonucleases based on the proximity of their genes to the genes of their cognate methylases. A similar method for identifying isoschizomers of known endonucleases, which isoschizomers possess a desired phys. property is also provided. Related methods for producing and cloning such endonucleases or other cytotoxic proteins are provided, as are several novel *M. jannaschii* restriction endonucleases. This method has been successfully employed and may be used to identify heretofore unknown restriction endonucleases as well as isoschizomers of known restriction endonucleases, such isoschizomers possessing a desired phys. property, such as thermostability. More specifically, in its broadest application the present invention comprises the following steps: (a) screening a target DNA sequence for the presence of known DNA methylase sequences and motifs characteristic of DNA methylases; (b) identifying open reading frames which lie close to the DNA methylase sequence of step (a); and (c) analyzing the protein product of the open reading frame of step (b) for endonuclease activity. Several novel restriction endonucleases isolated from *M. jannaschii* using the methods of the present invention are also provided, including MjaII, which is a thermostable isoschizomer of Sau961, MjaIII, which is a thermostable isoschizomer of MboI, and MjaIV, a new specificity recognizing GTNNAC. Also provided by the present invention is a novel method for stably cloning DNA sequences which might otherwise be unstable because the products encoded are toxic. One example provided has a stable, inducible clone encoding the normally toxic restriction

endonuclease *PacI* in the absence of a protective methylase. Restriction methylase.

L15 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2005 ACS on STN

2003:492541 Document No. 139:64335 Method for screening restriction endonucleases from *Methanococcus jannaschii*. Roberts, Richard J.; Byrd, Devon R.; Morgan, Richard D.; Patti, Jay; Noren, Christopher J. (New England Biolabs, Inc., USA). U.S. Pat. Appl. Publ. US 2003119027 A1 20030626, 25 pp., Cont.-in-part of U.S. Ser. No. 486,356. (English). CODEN: USXXCO. APPLICATION: US 2002-208557 20020730. PRIORITY: US 1997-PV57873 19970902; WO 1998-US18124 19980901; US 2000-486356 20000225.

AB A method is provided for identifying a restriction endonuclease, which includes the steps of (a) screening a target DNA sequence for the presence of known methylase sequence motifs, (b) identifying any open reading frames which lie close to the methylase sequence motifs screened in step (a), and (c) assaying the protein products of these open reading frames for restriction endonuclease activity. More specifically, genes containing DNA methylase motifs identified from restriction enzyme and methylase gene sequence (RM) databases are identified from available *M. jannaschii* GenBank database, and cloned and expressed using in vitro transcription/translation system for enzymic activity characterization. Methods for identifying isoschizomers of known restriction endonucleases, which isoschizomers possess a desired phys. property, such as thermostability, are also provided by the present invention, as are several novel restriction endonucleases isolated from *M. jannaschii*, *MjaIII* (*MboI* isoschizomer), *MjaIV* and *MjaV* (*RsaI* isoschizomer). Addnl., a gene was identified that encoded a previously observed endonuclease activity, designated *MjaII*. Also provided by the present invention are vectors suitable for cloning a DNA sequence encoding a cytotoxic protein, via independent transcription promoters which may be selectively controlled by several conditions. In summary, *M. jannaschii* genes for five new restriction endonucleases *MjaI-V* and a putative RE are disclosed with the corresponding nucleotides identified from following known GenBank U67541 (nt4687-5355), U67585 (nt11380-12492), U67508 (nt5632-6504), U67573 (nt1748-2485), U67590 (nt9251-10129)=U67591 (nt767-74), and U67561 (nt9158-10258) resp. A method for producing these cytotoxic proteins using such vectors and in vitro rabbit reticulocyte transcription/translation system is also provided, as are stable clones of all above cloned *M. jannaschii* REs and pos. controls, such as *HhaI*, *SfiI*, *HindIII*, *PacI* and *NlaIII*.

=> S MBOI

L16 618 MBOI

=> S MBOI AND L4

618 MBOI

L17 340 MBOI AND L4

	L #	Hits	Search Text	DBs
1	L1	56992	RESTRICTION ADJ (ENZYME OR ENDONUCLEASE)	US- PGPUB; USPAT
2	L2	115	MMEI OR MME1 OR (MME ADJ (I OR 1))	US- PGPUB; USPAT
3	L3	112	L1 AND L2	US- PGPUB; USPAT
4	L4	36671	MOTIF	US- PGPUB; USPAT
5	L5	181	L4 WITH L1	US- PGPUB; USPAT
6	L6	4	L5 AND L3	US- PGPUB; USPAT
7	L7	8	MJAI	US- PGPUB; USPAT